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10/527,662	03/11/2005	Joel Vandekerckhove	BJS-4465-6	7309

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EXAMINER	
FOSTER, CHRISTINE E	
ART UNIT	PAPER NUMBER
1641	

NOTIFICATION DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

USPTOMail@traskbritt.com

Office Action Summary	Application No.	Applicant(s)	
	10/527,662	VANDEKERCKHOVE ET AL.	
	Examiner	Art Unit	
	Christine Foster	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 January 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-7 and 13-16* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-7 and 13-16* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *3/11/05, 2/22/08* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 1/21/2010 has been entered.

2. Claims 1-2 and 4 were amended. New claims 15-16 have been added. Accordingly, claims 1-7 and 13-16 are currently pending and subject to examination below.

Priority

3. Acknowledgment is made of the present application as a proper National Stage (371) entry of PCT Application No. PCT/EP03/50402, filed 9/11/2003. Acknowledgment is also made of applicant's claim for foreign priority under 35 U.S.C. 119(a)-(d) to Application No. 02078801.4, filed on 9/12/2002 in Europe.

Claim Rejections - 35 USC § 112

4. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 1-7 and 13-15 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which

was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The instant claims recite methods for isolating at least one specific interaction partner of a compound, wherein the compound "does not interact with a majority" of proteins and/or peptides in a complex mixture. See especially claims 1 and 15.

The claimed methods therefore invoke a genus of compounds that possess certain functional characteristics, namely the ability to interact with at least one protein or peptide but not with the majority of proteins and/or peptides in a complex sample.

The MPEP states that the purpose of the written description requirement is to ensure that the inventor had possession, as of the filing date of the application, of the specific subject matter later claimed. The MPEP lists factors that can be used to determine if sufficient evidence of possession has been furnished in the disclosure of the application. These include "level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention." MPEP 2163.

In the instant case, the specification discloses examples of suitable compounds, such as a Lys-containing peptide to be used to interact with G-actin via the catalytic action of a transglutaminase (published application, [0074]). In addition, the specification contemplates fluorosulfenylbenzyol adenosine (FSBA) in order to interact with ATP-binding proteins [00084].

The specification expresses that when analyzing a complex mixture of peptides, the vast majority of peptides is not conjugated to the compound [0032]. However, the specification fails

to disclose what structural features are responsible for imparting this desired property, and therefore fails to disclose any correlation between structure and function.

For a broad generic claim, the specification must provide adequate written description to identify the genus of the claim. The MPEP further states that if a biomolecule is described only by a functional characteristic, without any disclosed correlation between function and structure of the sequence, it is "not sufficient characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence." MPEP 2163. The MPEP does state that for a generic claim the genus can be adequately described if the disclosure presents a sufficient number of representative species that encompass the genus. MPEP 2163. If the genus has a substantial variance, the disclosure must describe a sufficient variety of species to reflect the variation within that genus. See MPEP 2163. Although the MPEP does not define what constitute a sufficient number of representative species, the courts have indicated what do not constitute a representative number of species to adequately describe a broad generic. In *Gostelli*, the courts determined that the disclosure of two chemical compounds within a subgenus did not describe that subgenus. *In re Gostelli* 872, F.2d at 1012, 10 USPQ2d at 1618.

In the instant case, the specification discloses a number of species that would apparently interact only with a minority of peptides in certain complex mixtures. However, the specification does not adequately describe the genus of compounds having this property since it is not disclosed what structural features common to the genus are responsible for function. In particular, the specification does not identify what elements of the disclosed species are critical for maintaining this ability to interact only with a minority of peptides. As such, it is not possible to envisage what other compounds might possess the necessary functional characteristics. The

disclosure of a number of disparate chemical compounds does not in this case lead one of ordinary skill in the art to envisage the broad genus, as there is no disclosed common structure or other unifying theme that would serve to identify the members of the genus.

Regardless whether a compound is claimed per se or a method is claimed that entails the use of the compound, the inventor cannot lay claim to that subject matter unless he can provide a description of the compound sufficient to distinguish infringing compounds from non-infringing compounds, or infringing methods from non-infringing methods. University of Rochester v. G.D. Searle & Co., 358 F.3d 916, 926 (Fed. Cir. 2004).

In the instant case, it is further noted that the claimed "complex mixture" would encompass mixtures of varying compositions. As such, it is reasonable to assume that a compound might interact with a minority of the proteins and/or peptides in some complex mixtures, while interacting with the majority in other complex mixtures. Without reference to objective structural features common to the genus, it is not possible to determine what compounds fall within the claimed genus and what compounds do not (see rejection under § 112, 2nd paragraph below).

The claimed method is therefore analogous to the method claimed in University of Rochester and, just as in that case, "the inventor cannot lay claim to that subject matter unless he can provide a description of the compound sufficient to distinguish infringing compounds from non-infringing compounds, or infringing methods from non-infringing methods." University of Rochester, 358 F.3d at 926.

Because the specification does not disclose what compounds would have the desired characteristics, the claimed genus of compounds has not been adequately described.

6. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. Claims 1-7 and 13-15 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The instant claims recite methods for isolating at least one specific interaction partner of a compound, wherein the compound "does not interact with a majority" of proteins and/or peptides in a complex mixture. See especially claims 1 and 15.

The claimed methods therefore invoke a genus of compounds that possess certain functional characteristics, namely the ability to interact with at least one protein or peptide but not with the majority of proteins and/or peptides in a complex sample.

However, the claimed "complex mixture" would encompass mixtures of varying compositions. As such, it is reasonable to assume that a compound might interact with a minority of the proteins and/or peptides in some complex mixtures, while interacting with the majority in other complex mixtures.

In other words, the claimed functional characteristic depends on the composition of the sample, which may vary. For example, the specification discloses the example of FSBA, a compound that can form a complex with ATP-binding proteins [0084]. If one performed the claimed methods on a sample of ATP-binding proteins, this compound would presumably interact with all of the proteins. By contrast, if the sample did not contain any ATP-binding proteins, FSBA would presumably not interact with any of the proteins or peptides in the sample.

Without reference to objective structural features common to the genus, it is not possible to determine what compounds fall within the claimed genus and what compounds do not, since the inability of the compound to interact with the majority of proteins and/or peptides in the sample depends upon the identity of the sample, which may vary. See also MPEP 2173.05(b).

Claim Rejections - 35 USC § 102

8. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

9. Claim 16 is rejected under 35 U.S.C. 102(b) as being anticipated by Creighton, T.E. ("Proteins: Structures and Molecular Properties" Second Edition, W.H. Freeman and Company, New York, 1993), pages 10-20 and 31-41).

Creighton teaches diagonal techniques for the purification of peptides (i.e., specific interaction partners), in which those peptides in a peptide mixture (i.e., a complex mixture of molecules) that contain a particular amino acid are selectively isolated in two electrophoretic or chromatographic steps, which are performed with an intervening step modification step that alters the mobilities of modified peptides (see page 41). Specifically, the reference teaches adding a compound (e.g., iodoacetic acid, cyclohexanedione, or trifluoroacetyl, maleyl, or dinitrophenyl groups) to a complex mixture of peptides, wherein the compounds covalently modify specific amino acid residues in a peptide to form modified amino acid residues (see page

41 and also at pages 10-20 and 38-40, in particular at Equations 1.84, 1.22, 1.45, 1.79, 1.80, and 1.29).

Creighton further teaches performing a first separation step, which may be performed either by electrophoresis or by the more common high-pressure liquid chromatography (HPLC) analysis (page 41, right column, the second full paragraph; and left column, the first full paragraph).

After the first separation, fractions are subjected to an intervening modification so as to change the properties of the peptides which have been selectively modified by the compound. For example, the compound maleic anhydride can be added to modify lysine-containing peptides (page 41, right column and page 11, left column including Equation 1.22). After the first separation, the compound is removed (i.e., altered). As another example, the compound cyclohexanedione can be added to selectively modify arginine-containing peptides (page 41, right column and page 12, right column including Equation 1.29). After the first separation, arginine residues are regenerated by alkaline pH treatment (i.e., the cyclohexanedione compound is removed). Creighton et al. also contemplate selectively modifying cysteine-containing peptides, for example using iodoacetic acid and subsequent modification via performic acid (pages 17-20 and 41-42); as well as peptides containing methionine, histidine, and tryptophan (pages 17-18 and 41).

When the peptides are subjected to the same separation procedure a second time, peptides that have been modified by the compound will be isolated (see left column, the second full paragraph). As explained on page 41, third paragraph, when using HPLC chromatography, fractions must be modified and then reanalyzed (i.e., re-chromatographed). Similar techniques

can also be performed for chemical modification of a variety of amino acids using different compounds (page 41, right column; and pages 10-10).

With respect to the recitation that complexes containing the altered compound elutes at a different elution time as compared to complexes containing non-altered compound, Creighton makes clear that the mobilities of peptides that have been modified after the first separation are different in the second separation. Because the peptides have different mobilities, this is strong scientific evidence that the peptides would in fact elute at different elution times.

10. Claim 16 is rejected under 35 U.S.C. 102(b) as being anticipated by Cruickshank et al. ("Diagonal Chromatography for the Selective Purification of Tyrosyl Peptides", Canadian Journal of Biochemistry (1974) 52, 1013-17, of record).

Cruickshank et al. teach a method to isolate tyrosyl- or histidyl-containing peptides, comprising the steps of (a) adding a compound comprising a functional group that can be specifically altered (1-fluoro-2,4-dinitrobenzene, FDNB) to a complex mixture of molecules (carboxymethylated protein samples mixed with urea and other chemicals; see page 1014, right column) to form a compound-target complex (O-DNP-tyrosyl and DNP-histidyl-derivatized proteins, which are formed as a result of the reaction of FDNB with tyrosyl side chains to give the DNP group), (b) separating the resulting mixture by paper chromatography, (c) chemically altering the DNP compound by thiolysis, and (d) isolating at least one target molecule (tyrosine and/or histidyl-containing peptides) after DNP thiolysis by paper chromatography and final purification by electrophoresis. See entire selection, in particular the abstract; page 1013, right

column to page 1015, right column, first paragraph; Figures 1-2; and page 1016, the paragraph bridging the left and right columns.

Both chromatographic steps were performed by paper chromatography and the second step was performed "under identical conditions" with the earlier step (see page 1014, left column, "Experimental" and page 1015, "Isolation of Tyrosine-Containing Peptides", especially at the sentence bridging the left and right columns).

Claim Rejections - 35 USC § 103

11. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

13. Claims 1-7 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Creighton, T.E. ("Proteins: Structures and Molecular Properties" Second Edition, W.H. Freeman and Company, New York, 1993), pages 10-20 and 31-41) in view of Aebersold et al. (U.S.

6,670,194 B1) and in light of the evidence of Beals et al. ("Amino Acid Frequency" (1999), retrieved from http://www.tiem.utk.edu/bioed/webmodules/aminoacid.htm on 2/15/10).

Creighton is as discussed above, which teaches diagonal techniques for the purification of peptides (i.e., specific interaction partners), in which those peptides in a peptide mixture that contain a particular amino acid are selectively isolated in two electrophoretic or chromatographic steps, which are performed with an intervening step modification step that alters the mobilities of modified peptides (see page 41). Specifically, the reference teaches (a) adding a compound (e.g., iodoacetic acid, cyclohexanedione, or trifluoroacetyl, maleyl, or dinitrophenyl groups) to a complex mixture of peptides, wherein the compounds covalently modify specific amino acid residues in a peptide to form modified amino acid residues (see page 41 and also at pages 10-20 and 38-40, in particular at Equations 1.84, 1.22, 1.45, 1.79, 1.80, and 1.29).

Creighton further teaches (b) performing a first separation step, which may be performed either by electrophoresis or by the more common high-pressure liquid chromatography (HPLC) analysis (page 41, right column, the second full paragraph; and left column, the first full paragraph).

After the first separation, fractions are (c) subjected to an intervening modification so as to change the properties of the peptides which have been selectively modified by the compound. For example, the compound maleic anhydride can be added to modify lysine-containing peptides (page 41, right column and page 11, left column including Equation 1.22). After the first separation, the compound is removed (i.e., altered). As another example, the compound cyclohexanedione can be added to selectively modify arginine-containing peptides (page 41, right column and page 12, right column including Equation 1.29). After the first separation,

arginine residues are regenerated by alkaline pH treatment (i.e., the cyclohexanedione compound is removed). Creighton et al. also contemplate selectively modifying cysteine-containing peptides, for example using iodoacetic acid and subsequent modification via performic acid (pages 17-20 and 41-42); as well as peptides containing methionine, histidine, and tryptophan (pages 17-18 and 41).

When the peptides are (d) subjected to the same separation procedure a second time, peptides that have been modified by the compound will be isolated (see left column, the second full paragraph). As explained on page 41, third paragraph, when using HPLC chromatography, fractions must be modified and then reanalyzed (i.e., re-chromatographed). Similar techniques can also be performed for chemical modification of a variety of amino acids using different compounds (page 41, right column; and pages 10-10).

The compounds taught by Creighton possess the functional limitations claimed because they are capable of reacting with a functionality present in the interacting peptides. For example, cyclohexanedione as taught by Creighton is capable of reacting with the arginine-containing peptides as discussed above. Because these two moieties react in a specific manner, the chemical structure of cyclohexanedione may also be said to determine the specific interaction thereof. In addition, the compounds are altered after the first separation as discussed above. With respect to the recitation that complexes containing the altered compound elute at a different elution time as compared to complexes containing non-altered compound, Creighton makes clear that the mobilities of peptides that have been modified after the first separation are different in the second separation. Because the peptides have different mobilities, this is strong scientific evidence that the peptides would in fact elute at different elution times.

The teachings of Creighton differ from the instantly claimed invention in that the reference is silent as to whether the compounds used in the diagonal techniques **do not interact with the majority of proteins and/or peptides** in the complex mixtures. The reference makes clear that the compounds selectively interact with peptides containing particular amino acids, but is silent regarding the proportion of peptides in the mixtures that would actually interact with the compound.

Those of skill in the art at the time of the instant invention recognized the value in performing large-scale analyses of proteins in a so-called "proteomics" approach.

For example, Aebersold et al. discuss the recognized importance of proteins in biological processes. Like large-scale genomic analysis, global analysis of proteins expressed in a cell or tissue is also essential to describe a biological system (column 1, lines 20-60). Such complex samples can be analyzed in order to identify proteins in the context of disease states (column 3, lines 8-46). Techniques to assay proteins expressed in complex samples (such as blood, cells, tissues, and fractions thereof) are therefore desirable (see column 1, line 60 to column 3, line 45). It is noted that the instant specification indicates that the proteome is a complex mixture of proteins that in most cases has 100 different proteins or more (page 23, lines 26-35). Further, it is disclosed that a proteome is present in such samples as intact cells, lysate, biological fluid, etc. (ibid). Because these same samples are taught by Aebersold et al., it is presumed that they would necessarily contain 100 different proteins or more.

In addition, Aebersold et al. also teach simultaneous "multiplex" analysis of multiple proteins or of multiple samples in a single analysis (see column 6, lines 40-45; column 7, lines 37-42). For example, sequence identification of multiple peptide components of a protein mixture can be obtained in a single analysis (see also heading to Table 2).

To accomplish this goal of assaying proteins in complex samples, Aebersold et al. set forth a basic approach to analyze and detect specific proteins in complex samples (see especially at column 2, lines 49-57). Their approach involves selectively labeling and isolating specific peptide fragments from complex mixtures, and then characterizing the isolated peptides by mass spectrometric techniques (column 3, line 39 to column 7, line 42).

More particularly, Aebersold et al. teaches digesting labeled protein samples with proteases to produce peptide fragments prior to analysis by mass spectrometry (column 3, lines 39-68; column 5, lines 33-60; column 12, lines 44-53). This is done by adding a labeling reagent to a complex protein mixture, which is then cleaved into peptide fragments; peptide fragments that are labeled are then isolated and identified (ibid). By isolating and analyzing the isolated peptide fragments, the presence of protein(s) in the sample can be determined since the peptides are characteristic of the originating protein (column 3, lines 39-68). For example, sequence identification of multiple peptide components of a protein mixture can be obtained in a single analysis (see also heading to Table 2). Since the resulting peptide fragments are characteristic of the presence of the protein from which they originated, isolation and characterization of the peptide fragments can be used to determine the presence of the protein in the complex mixture (see also the abstract).

Similar to the labeling of specific amino acids such as cysteine as taught by Creighton, the labeling methods of Aebersold et al. also involve the use of compounds that have specific reactivity for certain protein groups, such as for sulfhydryl groups on cysteines (see Aebersold et al. for example at column 4, lines 27-39; column 6, lines 49-59; column 10, lines 30-43; column 15, line 54 to column 16, line 24). Next, proteins in the labeled sample are cleaved into peptides, and the labeled peptides are selectively isolated using chromatography procedures (see Aebersold et al. at column 5, lines 44-51; column 7, lines 10-15). Finally, labeled peptides are characterized by mass spectrometric techniques, e.g. to determine their amino acid sequence and identify the originating protein (column 3, line 39 to column 7, line 42 and especially at column 4, lines 54-60; see also at column 12, line 62 to column 13, line 65; and at columns 36-38).

The methods of Aebersold et al. are therefore highly analogous to those of Creighton in that they involve selectively labeling certain protein groups (e.g., cysteines) and then isolating peptides that have been labeled. In particular, the affinity labeling reagent of Aebersold et al. may react with sulfhydryl groups, which is highly analogous to the labeling methods of Creighton et al., which encompass sulfhydryl-reactive labeling of cysteine-containing peptides.

Therefore, it would have been obvious to one of ordinary skill in the art to perform the diagonal chromatography techniques of Creighton on complex samples such as blood, cells, tissues, and fractions thereof (as taught by Aebersold et al.).

One would be motivated to apply the diagonal chromatographic techniques to the analysis of complex mixtures (such as blood or cells) in order to conduct large-scale proteomic analysis, in view of the teachings of Aebersold et al. that the large-scale analysis of proteins

(proteomics) is essential in order to completely describe a biological system. More generally, one would be motivated to analyze very complex samples such as blood, cells, or tissue as taught by Aebersold et al. because Aebersold et al. taught that analysis of proteomes can be used to identify proteins whose expression level is changed in response to various disease states. Therefore, one would be motivated to study complex biological samples in order to identify proteins that play a role in disease. More generally, when taken together with the teachings of Aebersold et al. that the large-scale analysis of proteins expressed in a cell or tissue is important for completely describing a biological system, one would have been motivated to analyze samples containing as many proteins as possible in order to obtain as much information as possible about a particular biological system.

Consequently, it would have been obvious to arrive at the claimed invention by modifying the methods of Creighton in order to analyze more complex samples such as blood, cells or tissue (as taught by Aebersold et al.). When analyzing such complex samples, it would have been further obvious to first treat the protein-containing samples with a protease as taught by both Creighton and Aebersold et al., thereby cleaving proteins therein to form a protein peptide mixture. In particular, when taken together with the teachings of Aebersold et al. one would be motivated to digest the sample into peptide fragments as a necessary step prior to analysis by mass spectrometry, so that the presence of the protein from which the fragments originated could be identified. Motivation to combine the reference teachings in this manner also comes from the teachings of Creighton, since the diagonal chromatography procedures are performed on a "mixture of peptides".

One would have a reasonable expectation of success in employing the diagonal chromatography procedures of Creighton to analyze complex samples because the teachings of Aebersold et al. indicate that proteins which have been selectively labeled and isolated from complex mixtures can be successfully analyzed according to known methods, namely by mass spectrometry. Consequently, one of ordinary skill in the art would expect success in applying the methods of Creighton to analyze complex samples such as blood in conjunction with mass spectrometry analysis of the isolated, labeled peptides.

In addition, because Aebersold et al. also teach that their methods are compatible with any fractionation methods that reduce the complexity of the sample (column 16, lines 20-24), one of ordinary skill in the art would have had a reasonable expectation of success in analyzing complex mixtures fractionated by the methods of Creighton by the mass spectrometry techniques of Aebersold et al.

Returning to the limitation that the compound "does not interact with the majority of…proteins and/or peptides", as noted above Creighton teaches labeling of specific amino acid residues, such as cysteine- or arginine- containing peptides. As noted above, Creighton is silent as to whether peptides that contain these amino acids would be expected to represent the majority or the minority of the peptides in the sample.

However, amino acids exist in proteins at different frequencies. While some amino acids are abundant, others are relatively rare. As a result, selectively targeting only those peptides that

contain certain amino acids (such as cysteines) necessarily means that the compound would not bind to all of the peptides. This is explained by Aebersold et al. at column 14, lines 45-53:

> [T]agging and selective enrichment of cysteine-containing peptides significantly reduced the complexity of the peptide mixture generated by the concurrent digestion of six proteins. For this protein mixture, the complexity was reduced from 293 potential tryptic peptides to 44 tryptic peptides containing at least one cysteinyl residue.

See also at column 15, lines 58-66:

> The method as applied using a sulfhydryl reactive reagent significantly reduces the complexity of the peptide mixtures because affinity tagged, cysteine-containing peptides are selectively isolated. For example, a theoretical tryptic digest of the entire yeast proteome (6113 proteins) produces 344,855 peptides, but only 30,619 of these peptides contain a cysteinyl residue. Thus, the complexity of the mixture is reduced, while protein quantitation and identification are still achieved.

Thus, Aebersold et al. provides compelling evidence that of the peptides generated by digestion of proteins in a sample (e.g., digestion of a proteome), those containing cysteines would be in the **minority**, as this amino acid is "relatively rare".

Consequently, when employing the diagonal techniques of Creighton et al. to analyze peptides obtained from complex mixtures such as blood, it would seem that the compounds of Creighton et al. would necessarily interact only with a **minority** of the peptides present in such complex samples. In particular, in view of the evidence of Aebersold et al. that cysteines is a "relatively rare" amino acid, the evidence of record suggests that the selective modification of cysteines residues, as taught by Creighton, would necessarily result in the compound reacting only with a minority of the total peptides present in blood or other complex samples. For example, when employing iodoacetic acid to modify cysteines-containing peptides as taught by Creighton et al. in digested peptides obtained from complex samples such as blood (as taught by

Aebersold et al.), it is presumed absent evidence to the contrary that this compound would covalently modify only a minority of the digested peptides.

In addition, Creighton et al. contemplates targeting not only cysteine-containing peptides but also those peptides containing other amino acids such as arginine, lysine, methionine, histidine, and tryptophan, as discussed above.

Beals et al. provide evidence that cysteine has an observed frequency of 3.3% in vertebrates (see table). The frequency of arginine is comparable (4.4%), while methionine, histidine, and tryptophan occur at even lower frequency (1.8%, 2.9%, and 1.3%, respectively).

As noted above, the teachings of Aebersold et al. provide strong scientific evidence to suggest that in a peptide digest of a complex sample, those peptides containing cysteine would be in the minority due to the fact that this amino acid is relatively rare. When taken together with the evidence of Beals et al., similar results would be expected with arginine, lysine, methionine, histidine, and tryptophan, as these amino acids also occur at relatively low frequency.

Consequently, when employing the various amino acid-reactive compounds taught by Creighton et al. (such as iodoacetic acid, cyclohexanedione, or trifluoroacetyl, maleyl, or dinitrophenyl groups) in order to modify peptides in digested peptides from complex samples such as blood (as taught by Aebersold et al.), there is a strong scientific basis to believe that these compounds would covalently modify a minority of the peptides because they target amino acids that are relatively rare.

For all of these reasons, when employing the diagonal chromatography methods of Creighton to analyze complex samples such as blood, absent evidence to the contrary it appears that by performing the prior art methods, it would necessarily follow that the compound would not react with the majority of proteins and/or peptides in the sample.

With respect to claim 2, Creighton teaches a "mixture of peptides" as discussed above, while Aebersold et al. teaches samples such as blood, cells or tissue (i.e., complex mixtures of proteins).

With respect to claims 3-4, it is acknowledged that Creighton fails to specifically teach adding the compound to a complex protein mixture that is then *cleaved* into a protein peptide mixture prior to separation step (b). Rather, in Creighton the compound is added to a protein peptide mixture; there is no specific teaching of a step in which the peptides are initially obtained by cleavage of proteins.

However, Aebersold et al. also teaches digesting labeled protein samples with proteases to produce peptide fragments prior to analysis by mass spectrometry (column 3, lines 39-68; column 5, lines 33-60; column 12, lines 44-53). In particular, Aebersold et al. exemplify adding a labeling reagent to a complex protein mixture, which is then cleaved into peptide fragments; peptide fragments that are labeled are then isolated and identified (ibid). By isolating and analyzing the isolated peptide fragments, the presence of protein(s) in the sample can be determined since the peptides are characteristic of the originating protein (column 3, lines 39-68). For example, sequence identification of multiple peptide components of a protein mixture can be obtained in a single analysis (see also heading to Table 2). Since the resulting peptide

fragments are characteristic of the presence of the protein from which they originated, isolation and characterization of the peptide fragments can be used to determine the presence of the protein in the complex mixture (see also the abstract).

As discussed above, the affinity labeling reagent of Aebersold et al. (which may react with sulfhydryl groups) is highly analogous to the iodoacetic acid modifying reagents of Creighton et al., which may also be sulfhydryl reactive.

Therefore, when performing the method of Creighton in order to analyze complex samples such as blood, cells or tissue (as taught by Aebersold et al.), it would have been obvious to treat the samples with a protease as taught by Aebersold et al. (thereby cleaving proteins therein to form a protein peptide mixture) as a necessary step prior to analysis by mass spectrometry. In particular, one would be motivated to digest the sample into peptide fragments so that the presence of the protein from which the fragments originated could be identified. Although Creighton exemplifies labeling a mixture of peptides, it would have been obvious to first label the complex mixtures of proteins (e.g., blood, cells, tissue) taught by Aebersold with the labeling compound of Creighton and to subsequently digest the sample into a protein-peptide mixture because this order is exemplified in the analogous methods of Aebersold et al. In addition, the selection of any order of performing process steps is prima facie obvious in the absence of new or unexpected results. See MPEP 2144.04.

Motivation to combine the reference teachings in this manner also comes from the teachings of Creighton, since the diagonal chromatography procedures are performed on mixtures of peptides.

With respect to claims 5-7, Aebersold et al. teach that isolated peptides can be characterized by mass spectrometric techniques: in particular, the sequence of isolated peptides can be determined using mass spectrometry techniques, and by application of sequence database searching techniques, the protein from which the sequenced peptide can be identified using the mass spectrometry data (which measures the peptide masses). See column 3, lines 54-60; and columns 13-14.

Therefore, when performing the peptide isolation method of Creighton on a complex protein mixture in order to identify proteins in the mixture (as taught by Aebersold et al.), it would have been further obvious to one of ordinary skill in the art at the time of the instant invention to identify the isolated peptides by mass spectrometry in combination with sequence database searching as taught by Aebersold et al. because Aebersold et al. taught that isolated peptides can be sequenced and characterized by mass spectrometry in this manner, thereby allowing identification of the protein from which they originate, and consequently allowing for determination of the presence of that protein in the complex mixture.

14. Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Creighton in view of Aebersold et al. and in light of the evidence of Beals et al. as applied to claim 1 above, and further in view of the evidence of Sahasrabudhe (U.S. 5,705,351) or Chang (U.S. 5,474,780).

Creighton is as discussed above, which teaches compounds including fluoro-2,4,-dinitrobenzene and maleic anhydride to modify specific amino acid residues (see especially page 41, right column; page 31, right column; and page 11, left column). However, the reference is silent as to whether such compounds are drugs.

Sahasrabudhe provides evidence that fluoro-2,4-dinitrobenzene is a drug[1] in that it can be used to chemically treat cells for therapy of non-leukemic cancer (column 3, line 34 to column 4, line 16; column 17, lines 29-49). In addition, fluoro-2,4-dinitrobenzene can be used in diagnosis, e.g. to identify patients at risk of cancer or to monitor therapy in cancer patients (column 7, line 31 to column 8, line 2; column 14, line 31 to column 15, line 28).

Chang teaches that maleic anhydride is used as an ingredient in medical preparations for drug delivery (abstract and column 3, lines 1-21).

Therefore, in light of the evidence of Sahasrabudhe and Chang, the teachings of Creighton and Aebersold et al. meet the claim as the compounds taught by Creighton are drugs.

15. Claim 14 is rejected under 35 U.S.C. 103(a) as being unpatentable over Creighton in view of Aebersold et al. and in light of the evidence of Beals et al. as applied to claim 1 above, and further in view of GE Healthcare ("Fraction Collectors: Frac-950 and Frac-920", Data File 18-1153-57 AD (May 2001), retrieved from http://www1.gelifesciences.com on 4/8/09).

Creighton and Aebersold et al. are as discussed above. Creighton teaches HPLC chromatography but fails to provide details regarding the specific procedures to be used. Therefore, the references fail to specifically teach pooling fractions to avoid elution overlap between different peaks.

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[1] A "drug" is defined in the art as "a substance intended for use in the diagnosis, cure, treatment, or prevention of disease"; "a substance that has a particular effect on the body" (Penguin English Dictionary); "any substance used as an ingredient in medical preparations"; "any substance that affects the normal body functions" (Collins Dictionary of Biology).

However, it was known in the art to adjust the size of collected fractions when performing chromatographic procedures in order to avoid re-mixing of proteins separated on the column. See GE Healthcare at column 3, "Collect the fractions you want", where it is discussed that while too many fractions will make for too much work (many tubes), while a fraction size that is too large will result in loss of resolution as peaks separated on the column will be re-mixed in the collected fractions. This is done by pooling multiple drops (i.e., fractions) into tubes (i.e., a plurality of pooled fractions). GE Healthcare teaches that by using automated peak fractionation in this manner, peak overlap can be reduced (Figure 7).

Therefore, it would have been obvious to one of ordinary skill in the art to employ automated peak fractionation, thereby pooling fractions having distinct elution times in such a way as to reduce peak overlap, because GE Healthcare taught that such procedures achieve the best results in chromatography (which is the technique employed by Creighton).

Response to Arguments

16. Applicant's arguments filed 1/21/2010 have been fully considered.

17. With respect to the rejections of claims 1-7 under § 103 as being unpatentable over Creighton in view of Aebersold et al., Applicant argues that the references do not teach that the compound "does not interact with the majority of said proteins and/or peptides" (Reply, pages 7-8).

In particular, Applicant argues that in Creighton, only one protein is analyzed. Applicant also points to Aebersold et al. at column 16, in which it is discussed that a small percentage of proteins contain no cysteine residues.

This is not found persuasive because as discussed in detail above, both Creighton and Aebersold et al. first digested their protein-containing samples prior to addition of amino acid-reactive compound. As such, while Creighton analyzed only one protein, a mixture of a plurality of peptides was in fact being analyzed (the digested protein). Similarly, Aebersold et al. directs the skilled artisan to first digest the complex sample into peptides. Therefore, while only a minority of intact proteins may lack cysteine residues, the issue at hand is the proportion of cysteine-containing peptides in a digested peptide mixture, such as a digested proteome or other complex mixture as taught by Aebersold et al.

Aebersold et al. elsewhere make clear (columns 14-15) that such cysteine-containing peptides would in fact be in the **minority** in such mixtures because of the fact that cysteine residues are relatively rare; and Applicant has not advanced evidence to the contrary. Similarly, because other amino acids such as methionine (also contemplated by Creighton for selective labeling) are also known to be relatively rare (as evidenced by Beals et al.), the evidence of record also suggests that when studying a peptide digest from complex samples, it would also necessarily follow that compounds reacting with these other low frequency amino acids would also react with the minority, rather than the majority, of peptides in the sample.

18. Regarding new claim 16, Applicant argues for patentability on the above grounds (Reply, page 9); however, it is noted that the new claim does not recite the limitation that the compound "does not interact with the majority of said proteins and/or peptides".

Applicant further argues for patentability of claim 16 on the basis of the claim terminology "specifically interacts" (pages 9-10).

In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., one that does not occur uniformly throughout the sample) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

The instant specification discloses that:

> The binding of a compound to the target is specific, meaning that said compound binds to at least one molecule in a complex mixture of molecules and not to other molecules.

Such a definition fails to exclude the compounds of Creighton, which specifically interact with certain amino acids and not others. As made clear by Aebersold et al. in columns 14-15 as detailed in the rejection above, compounds that target cysteines interact with a minority of peptides in a peptide digest. In other words, such compounds bind to at least one molecule in a complex mixture of molecules and not to other molecules.

In addition, it is evident that the term "specifically interacts" does not mean that the compound exclusively interacts only with a single protein or peptide, as the compounds contemplated by the instant specification include those that bind to multiple proteins or peptides (such as to a class of peptides) [0032]. For all of these reasons, when the claims are given their broadest reasonable interpretation, there is nothing in the terminology "specifically interacts" that would require that the compound only interact with a precise number of molecules in the mixture.

19. With respect to the rejections of claim 14 under § 103 as being unpatentable over Creighton in view of Aebersold et al. and GE Healthcare, Applicant argues that GE Healthcare

relates to single fractions and does not concern pooling of multiple fractions, and more specifically does not concern pooling of non-neighboring fractions (Reply, pages 10-11).

Initially, it is noted that the claims fail to recite or require pooling of "non-neighboring fractions". Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims.

Moreover, the collecting of multiple drops into a single tubes as taught by GE Healthcare reads on the claimed limitation of pooling fractions to combine a plurality of fractions having distinct elution times into a plurality of pooled fractions. This is because each drop eluting at a different point in time may be considered a 'fraction' of the sample that is coming off the column, and GE Healthcare directs the skilled artisan to combine or pool multiple fractions or drops together into a single tube.

20. Applicant does not separately argue the limitations of dependent claim 13 (Reply, page 10).

Conclusion

21. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Conrads et al. ("Utility of Accurate Mass Tags for Proteome-Wide Protein Identification" Anal. Chem. 2000, 72, 3349-3354) provides evidence that of the 918,655 possible tryptic polypeptides within *C. elegans*, only 124,668 contain cysteines residues (page 3354, left column, first paragraph). The reference is therefore being cited for providing evidence cumulative to that of Aebersold et al., to show that compounds that selectively target cysteine-containing peptides would be expected to react with a *minority* of peptides in a peptide digest.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Foster whose telephone number is (571) 272-8786. The examiner can normally be reached on M-F 6:30-3:00. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mark Shibuya, can be reached at (571) 272-0806. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Christine Foster/
Examiner, Art Unit 1641